Assessment of Genetic Variation between Coastal Steelhead Populations Associated with Different Run-timings

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Abstract

Seven microsatellite loci were used to investigate genetic relationships among three winter-run, two summer-run, and one hatchery population of steelhead sampled through the mainstem Klamath River. Multiple classifications for steelhead exist on the Klamath River including different adult run-timings, reproductive ecotypes, and life history characteristics. This study attempted to assess the genetic variation between summer and winter run steelhead to determine if these fish migrating through the Lower Klamath River constituted discrete reproductive populations or a single panmictic population. Samples compared between 2000 and 2001 were taken from both wild and hatchery fish below Weitchepec, California. Overall, population heterogeneity was high in all samples. All populations were out of Hardy-Weinberg proportions for at least one locus and significant linkage disequilibrium was found, suggesting the existence of multiple independent populations in each collection. Additionally, a majority of pair-wise F_{ST} comparisons suggested low, yet significant, genetic variability between run-timings (5 of 6 samples) and also between the hatchery population and putative wild steelhead samples from two of the years. These results contrast with other genetic studies on sympatric steelhead populations, since they supported closer genetic lineage between summer- and winter- run steelhead fish from the same basin than among all summer-run collections. These data are consistent with the last stock identification effort for steelhead in the KlamathRiver. The techniques and markers used in this study could be used to develop a better understanding for the relationship of steelhead and trout populations in the Klamath Basin.

Introduction

The Klamath River Basin encompasses two distinct western subbasins of North America prior to reaching the Pacific Ocean near Klamath, California. As it flows from its headwaters in Southern Oregon's high desert mountains to Northern California's temperate redwood rainforests, it covers a broad heterogeneous landscape. The river supports the greatest number of coastal steelhead (*Oncorhynchus mykiss irideus* (Behnke 1992)) in California (McEwan et al. 1996). In the Klamath basin, coastal steelhead have evolved multiple life history characteristics and reproductive strategies for persistence in a system where critical habitat parameters are highly variable.

The scale of reproductive isolation among stocks of Klamath River coastal steelhead is disputed (Table 1). The National Marine Fisheries Service (NMFS) recognizes two distinct reproductive ecotypes (Busby et al. 1996), based upon sexual maturation, that migrate into the basin during the summer or winter. An ecotype is a population of a species that is genetically adapted to distinct environmental conditions. The summer ecotype enters freshwater sexually immature, and requires several months for eggs to ripen to spawning condition (Burgner et al. 1992). Peak migration period in these fish is during June and the spawning season remains unknown. The winter ecotype matures sexually in the ocean and enters the river between November and March (Hopelain 2001) finding suitable spawning habitat relatively quickly, with a peak in spawning activity during January. A potential overlap in migration and spawning periods make differentiating these ecotypes difficult (Roelofs 1983). The existence of a third group of sexually mature steelhead migrates in the Klamath mainstem between July and October. Though these have been called fall run Hopelain (2001), they may be an extension of a summer ecotype. This run also contains a sexually immature steelhead called the half-pounder (Hopelain 2001, Kesner and Barnhardt 1972, Everest 1973). Half-pounders typically spend only 2-4 months in the estuary or nearshore, enter the river to overwinter, and return to the ocean for 1-2 years before spawning. Genetic information can often help clarify relationships among these various groups of river migrating adult fish, delineate reproductively independent populations, and identify genetic conservation units.

Genetic information can often help clarify relationships among these various groups of river migrating adult fish, delineate reproductively independent populations, and identify genetic conservation units. Microsatellites have proven to be a particularly useful class of DNA molecular markers for studying genetic variation in steelheads and other salmonids

(e.g. Angers et al. 1995, Banks et al. 2000, Garant et al. 2000, Neraas and Spruell 2001). High levels of polymorphism, potential availability of published information on primer sequences, and non-lethal sampling make this technique ideal for population genetic studies.

The present study was designed to describe the genetic variation associated with different run-timings of coastal steelhead entering the Klamath River Basin. The speicifc null hypothesis evaluated is that Klamath River steelhead from different run-timing periods are genetically similar and form a single population. These analyses assess the frequencies of alleles and genotypes within each collection of steelhead from different run-timings between the years 2000-2002.

Methods and Materials

Sample Collection - Scale and fin clip samples of 869 coastal steelhead (*O. m. irideus*) were collected by the Yurok Tribal Fisheries Program and dried in envelopes. These samples were collected between January 2000 and September 2002. All samples were taken below the Klamath-Trinity Rivers confluence, thus eliminating the ability to detect genetic variation attributable to Klamath-Trinity sub-basin spatial differences.

DNA Extraction - 238 samples were selected during two summer and three winter periods, based on their representation of putative run-timing populations (Table 2). These putative run-timings were based on information in Table 1, and in particular the classification of Busby et al. (1996). The rest of the samples were either during periods of outmigration or contributed to a sample that would have been too small in number to compare statistically. In a review of recent literature, an average of 40 samples is considered reasonable to delineate populations (Beacham et al. 2000; Banks et al. 2000). Samples were extracted from dried tissue using the Promega Wizard SV 96 Genomic DNA Purification System™. Hatchery fish were identified by lack of adipose fin. The only year that all samples had this information collected was 2002. Therefore, a portion of the 2000 and 2001 samples could be hatchery fish, but this is unknown since this data was not collected for each sample.

Microsatellite experiment - Twenty-two microsatellite DNA primer pairs previously developed for *Oncorhynchus* and *Salvelinus* species were screened using the Polymerase Chain Reaction (PCR,Table 3). Initially, amplification was attempted with a touchdown PCR, in which the annealing temperature decrease by one degree during the first ten cycles. PCR products (alleles) were run for one hour at 50 watts on 5.5% polyacrylamide gels, stained with a Fluorescein-Agarose overlay (Rodzen et al. 1998), and scanned with a Molecular Dynamics 595 FluorImager. Of the 22 primers pairs examined, seven (Table 3) were selected that were readily amplifiable, did not amplify multiple loci or produce difficult to interpret stutter bands, and did not contain null alleles. These seven polymorphic primer sets were optimized for use in this study by adjusting magnesium chloride ($MgCl_2$) concentration, initial annealing temperature, and DNA concentration. Optimized touchdown PCR conditions started with a 3min denaturing step at 94°C, followed by 10 cycles of 94°C for 45 sec, locus-specific starting annealing temperature (Table 3) for 45sec (decreasing 0.5°C/cycle) and 72°C for 1min; followed by 33 cycles of 94 °C for 30sec,

locus-specific annealing temperature (5° less than the starting annealing temperature) for 30sec, and 72°C for 1min; and a final extension at 60°C for 3min. Amplifications of all microsatellite loci were carried out in 10 μl reaction. These included 1 μl 10X PCR buffer, 1.5 or 0.75 μl 50 mM $MgCl_2$ depending on optimal conditions for each loci, 0.10 μg BSA, 0.80 μl 2 mM dNTP mixture, 0.6μM forward primer labeled with one of three fluorescent dyes (NED, VIC, or 6FAM), 0.60 μM unlabeled reverse primer, 0.075 μl FASTSTART *Taq* polymerase (0.375 U total), 1 or 0.50 μl DNA (approximately 50ng DNA total) according to the optimized primers conditions. Sterile dH_2O was added to reach the full 10μl volume. PCR products were diluted (Table 3) and separated electrophoretically on a 5.5% polyacrylamide gel using the MJ Research BaseStation gel analysis system (MJ Research, Inc., San Francisco, CA). Allele sizes were designated using a Rox-labelled Genescan 400 or 500 size standard (MJ Research, Inc.) run in each lane. Previously amplified products were included on each gel to ensure consistent scoring of individuals across all gels. Gel images were analyzed using MJ Research, Inc.'s Cartographer® software.

Population genetic analyses - Allelic frequency and heterozygosity was calculated with the software package TOOLS FOR POPULATION ANALYSIS (TFPGA 1.3, Miller 2003). Fisher exact tests for Hardy-Weinberg and genotypic pair-wise disequilibrium were performed with the software package GENETIC DATA ANALYSIS (GDA, Lewis and Zaykin 2002). Measuring pair-wise linkage disequilibiurm will evaluate the association of inherited alleles at different loci. High linkage disequilbrium and/or significant departures from Hardy Weinberg proportions are indications of non-random genetic assortment. P-values were estimated by 3200 random permutations setting the significance level (α) at 0.05. The software package GENEPOP ON THE WEB (GENEPOP) (Raymond & Rousset 1995) was used to calculate genotypic differentiation at each locus. Significance of observed differentiation was tested with an unbiased estimate of the *P*-value of a log-likelihood (*G*) based exact test (Goudet 1996).

A matrix of pairwise F_{ST} values was estimated between years and runs for determining degree of population differentiation (Weir and Cockerham 1984) with the software package GENETIX (Belkhir et al. 2000). The probability of each value's departure from the null hypothesis was computed following 2000 random permutations. F_{IS} (Nei 1978), a measurement of inbreeding and indicator of nonrandom mating, was computed in GDA where 1000 bootstrap resamplings yielded 95% confidence intervals to assess

statistical significance over loci. Hierarchical cluster analysis was determined using the UPGMA algorithm (Sneath and Sokal 1973) calculated using Nei's (1978) unbiased minimum distance in TFPGA 1.3 with 1,000 bootstrapped permutations. Nei's unbiased genetic distance (1978) was computed with GENETIX with a P-value based on 2000 permutations. A consensus UPGMA diagram was then generated with the original branch lengths, and all bootstrap values were plotted on to the dendrogram to indicate stability of the nodes. An Analysis of Moleculer Variance (AMOVA) was used to partition the allelic variance and determine divergence within and among populations with the software packet ARLEQUIN 2.0 (Excoffier et al. 1992). This program generates *F*-statistics analogous to the θ values of Wier and Cockerham (1984) and evaluates the significance of using exact *F* permutation procedures (Excoffier et al. 1992). Factorial Correspondence Analysis (FCA) was computed with GENETIX (Belkhir et al. 2000) to ordinate allele frequency distribution differences between three years of putative winter runs, two years of putative summer runs, and a year of hatchery stock. This computation is done by transforming the allele frequency data into a contingency table, where a Chi-squared distance measures the relatedness between any two samples' allele frequencies. The resulting factorial axes can be ordered by their largest eigenvalue. The mapping of a sample onto these axes can by used to express which samples are most different or similar for a given axis.

Results

Allele frequencies, genetic diversity, Hardy-Weinberg, Linkage Disequilibrium - Allele size and frequency, observed and expected heterozygosity under Hardy-Weinberg proportions, Nei's (1978) unbiased heterozygosity; and sample size (N) for seven loci are included in Table 4. Except for the summer 2001 sample, each collection contained at least one locus with heterozygosity levels that deviated from Hardy-Weinberg proportions (Table 5). OtsG 253c, a primer designed by the NMFS-Santa Cruz laboratory (C. Garza, personal communication), displayed the greatest deviation from within-locus disequilibrium (out of equilibrium in 4 of 6 collections), while the other six loci did not show any particular pattern of being out of Hardy-Weinberg proportions. Out of 147 pair-wise combinations, 65 had significant levels (44.2%,α=0.05) of linkage disequilibrium. When the same statistical analysis was used while preserving genotypes to remove within locus disequilibrium, pair-wise linkage disequilibrium was reduced in all collections except Winter 2001. Overall, 44

of 147 pair-wise comparisons (29.9%, α=0.05) showed significant departure from Hardy-Weinberg equilibrium suggesting additional sources of population structuring, not accounted for by the grouping identified as temporal samples. F_{IS} values ranged from 0.003 (Summer 2001) to 0.131 (Winter 2000). Significant departures from F_{IS} coefficient were detected in three collections (Table 6).

Population structure - Highly significant ($p<0.001$) G-tests of genotypic differentiation were observed at four loci and significant tests ($p<0.05$) at the other 3 loci between all six samples. Significant F_{ST} values for pair-wise comparisons were detected between each collection of winter and summer runs except 2001(Table 7). A comparison of these values with significance tests of Nei's unbiased genetic distance (1978) showed similar results (Table 8). Between years, significant F_{ST} values were not found for winter samples between 2000 and 2002 or summer samples from 2001 and 2002. The 2002 adipose-clipped sample collection was significantly different than all populations except summer 2002. AMOVA results attributed greater variance to differences among winter and summer runs in a single year (2.59%) than differences among both winter and summer samples combined between 2001 and 2002 (Table 9). The majority (97.9%) of allelic variance was attributed to individuals.

Computation of Nei's unbiased genetic distance over 1000 permutations was used for UPGMA cluster analysis and showed strong support for differentiation between summer and winter runs. However, differentiation among summer or winter runs from one year to the next was weak (Figure 1). FCA with GENETIX showed the largest component of variance explained 37.7% of the variation and the second component 19.2%, suggesting increased marker resolution may be neccesary to adequately differentiate populations.

Discussion and Conclusion

Tests of genotypic differentiation show statistically significant differences between samples. A majority of the pair-wise F_{ST} and Nei's genetic distance values supports small genetic differences existing between summer and winter run-timings. These data suggest that there exists weak structuring among summer and winter run-timings of steelhead in the Klamath River. The recognition of a distinct summer metapopulation of steelhead in the Klamath River as determined by KRSIC (1993) seems reasonable, although too broad for identifying stocks for managment and conservation purposes. Much greater sampling and/or additional marker development is neccesary to determine the geographic component of the Klamath steelhead's population structure. Increased sampling and genetic analysis of steelhead at a spatial scale, in their natal streams, will be required for identification of reproductively-isolated breeding populations.

Significant tests for departure from Hardy-Weinberg proportions at multiple loci within all five collections, and significant F_{IS} for three collections indicated non-random mating characteristics for these collections. Also, pair-wise linkage disequilibiurm evaluated the association of inherited alleles at different loci, and the high linkage disequilbrium observed is indicative of non-random genetic assortment. Possible causes of these features may include inadequate sampling, population bottlenecks, and stock admixture. Stock admixture is likely given the number of breeding populations recognized by the Klamath River Stock Identification Committee (KRSIC, 1993). The lower average number of alleles per locus for the summer-run and hatchery population suggests there is potential that population size reduction (bottlenecking) may be a cause for these results.

The only sample known to be the product of a single reproductively isolated population were adipose-clipped hatchery fish collected in 2002. This collection's significant differentiation from all samples except the wild summer 2002 collection, suggests hatchery stocks are isolated from the majority of other stocks. However, since they were not significantly different from the summer 2002 collection, it suggests that hatchery steelhead can stray into wild stocks in certain years. The 2002 hatchery and wild steelhead may not be significantly different because straying hatchery steelhead migrating upstream in the summer may have interbred with wild summer fish, thus producing genetically similar progeny which were sampled. No hatchery adipose-clipped steelhead were noted in the 2001 putative summer-run sample collection.

AMOVA results suggest that there is significant allelic variance between run-timings and among individuals. The differences between run-timings (2.69%) are considerably

larger than the nonsignificant variance attributable to differences between years. In comparison, Nielsen and Fountain's (1999) results from the Middle Fork of the Eel River showed greater overall allelic variance (18.3%) was attributable to interannual variance than among samples identified as distinct run-timings. The UPGMA dendrogram supports differentiation between winter and summer run-timings being highly supported compared to less, poorly supported differentiation among the three winter- or two summer-run samples.

GENETIX'S FCA shows that winter runs cluster with increased proximity to each other than summer runs from different years. One possible explanation for this is greater geographic isolation among summer-run than winter-run fish. When summer steelhead enter the Klamath mainstem they spend the majority of time isolated in subbasins maturing. When the spawning run occurs they are already isolated, limiting gene flow between summer populations. It is possible that there is more gene flow between winter-runs since spawning fish enter sexually mature and migrate together upstream with limited geographic isolation among mature fish and increased straying among stocks. The increased genetic simiarity between winter runs is observable in the FCA graph where the winter run collections appear more proximate. An alternative explanation for the separation of summer-run steelhead may be that their survival is mainly influenced by in-river conditions and undergo differential survival, causing increased bottlenecking among summer-run stocks, and increased differences among these collections. Mainstem summer water conditions may influence survival of salmonids oversummering or migrating through the Klamath to coldwater refugias.

Data from thus study show some congruence with the currently recognized population structure of steelhead (KRSIC 1993). It supports the managment of summer-run steelhead as a metapopulation, with gene flow between summer-run stocks likely greater than with winter-run stocks in the same subbasin. These data demonstrate that there exists slight, yet significant differences between winter and summer runs. These data also point to the paucity of genetic stock information known about steelhead on a basinwide geographic scale and suggest additional studies are neccesary to fully understand the genetic diversity of steelhead populations in the Klamath River basin. Analysis of genetic samples from adult steelhead collected in different Klamath-Trinity River subbasins would likely identify the distinctiveness of multiple isolated summer- and winter-run spawning populations. Also, additional sampling of steelhead at the Irongate and Trinity hatcheries would provide insight into apparent introgression by hatchery stocks

into wild steelhead. A study comparing anadromous steelhead to resident trout populations above Iron Gate dam may provide important information about the relationship of downstream wild and hatchery fish to these populations, and assist in evaluating the impacts on the loss of connectivity due to Iron Gate dam.

Table 1. Classification of different run-timings and reproductive ecotypes of steelhead found in the Klamath River basin.

Steelhead race	KRSIC (1993)	Hopelain (1998)	USFWS (1979)	Busby et al.(1996)	Moyle (2002)
Spring/Summer	May- July	March-June	April-June		April- June
Fall	August- October	July-October	August-November		
Winter	November- February	November-March	November-February		November-April
Stream-maturing				April- October	
Ocean-maturing				September-March	

Table 2. Monthly collection sizes for steelhead collected in the Lower Klamath River below Weitchepec, CA. Bolded samples in parentheses were used in the study.

	2000	2001	2002
January	**49 (11)**	**56 (20)**	**79 (13)**
February	**33 (15)**	**132 (25)**	**65 (14)**
March	**50 (16)**	80	**39 (13)**
April	2	15	3
May	1	**2 (2)**	**14 (14)**
June	2	**3 (3)**	**50 (49)**
July	6	**10 (6)**	**33 (4)**
August	6	**21 (12)**	0
September	6	**11 (9)**	1
October	0	6	0
November	0	4	4
December	0	**85 (12)**	1

Table 3: Twenty-two primers pairs evaluated in this study, their variability, PCR imaging dilution, and starting annealing temperature (T_A) for the screening touchdown PCR reaction. Those primer names in bold were selected for further optimization.

Primer	Variability	PCR dilution	Source	T_A (°C)
Ots104	Monomorphic		Nelson and Beacham (1999)	55
OtsG401	Polymorphic		Williamson et al. (2001)	65
OtsG249	Did not amplify		"	60
OtsG3	Polymorphic		"	60
OtsG85	**Polymorphic**	**1:60**	**Williamson et al. (2001)**	**57**
OtsG253c	**Polymorphic**	**1:40**	**C. Garza, per. Comm.**	**68**
OtsG83b	**Polymorphic**	**1:32**	**Williamson et al. (2001)**	**65**
Ots103	Polymorphic		Beacham et al. (1998)	62
OtsG243	Polymorphic		Williamson et al. (2001)	62
OtsG249b	**Polymorphic**	**1:48**	**Williamson et al (2001)**	**57**
Ots2	Monomorphic		Banks et al. (1999)	50
Ots3	Polymorphic		"	52
Ots4	Did not amplify		"	52
One11b	Polymorphic		Scribner et al. (1996)	62
Omy27	Polymorphic		M. O'Connell, per. comm.	60
Omy1101	**Polymorphic**	**1:32**	**M. O'Connell, per. comm.**	**62**
Omy77	Polymorphic		Morris et al. (1996)	57
Omm1082	**Polymorphic**	**1:64**	**Rexroad et al. (2002)**	**57**
Omm1087	**Polymorphic**	**1:32**	"	**57**
Ssa85	Monomorphic		O'Reilly et al. (1996)	57
Ssa289	Polymorphic		McConnell et al. (1995)	55

Table 4. Allele sizes (in bp), allele frequencies, observed heterozygosities (H_o), expected heterozygosities unbiased (H exp) and not unbiased (H n.b.) for each population in each locus.

LOCUS	ALLELE SIZE	POPULATION					
		Winter 2000	Winter 2001	Summer 2001	Winter 2002	Summer2002	Hatchery2002
OTSG83b							
	92	0.012	0.013	0.000	0.000	0.040	0.000
	96	0.012	0.013	0.000	0.015	0.013	0.039
	100	0.073	0.081	0.016	0.061	0.092	0.154
	104	0.012	0.105	0.081	0.076	0.145	0.077
	108	0.122	0.093	0.097	0.106	0.092	0.000
	112	0.085	0.151	0.048	0.03	0.092	0.077
	116	0.049	0.047	0.032	0.076	0.079	0.058
	120	0.134	0.047	0.097	0.03	0.053	0.115
	124	0.061	0.023	0.048	0.091	0.040	0.115
	128	0.061	0.035	0.048	0.136	0.066	0.058
	132	0.037	0.093	0.113	0.061	0.092	0.077
	136	0.085	0.058	0.081	0.061	0.105	0.115
	140	0.085	0.093	0.081	0.061	0.053	0.019
	144	0.073	0.035	0.081	0.076	0.013	0.039
	148	0.037	0.023	0.048	0.046	0.000	0.000
	152	0.024	0.012	0.016	0.015	0.000	0.000
	156	0.012	0.000	0.065	0.015	0.000	0.000
	160	0.012	0.070	0.016	0.030	0.000	0.000
	164	0.012	0.012	0.016	0.000	0.000	0.039
	168	0.000	0.000	0.000	0.000	0.026	0.019
	172	0.000	0.000	0.016	0.015	0.000	0.000
	H exp.	0.921	0.917	0.927	0.924	0.914	0.907
	H n.b.	0.932	0.928	0.942	0.939	0.926	0.925
	H obs.	0.854	0.907	0.968	0.849	0.711	0.769
OMM1087							
	238	0.048	0.023	0.000	0.029	0.000	0.000
	242	0.012	0.035	0.031	0.014	0.013	0.019
	246	0.0605	0.047	0.063	0.043	0.075	0.000
	250	0.214	0.198	0.172	0.086	0.138	0.135
	254	0.286	0.209	0.172	0.229	0.200	0.231
	258	0.060	0.058	0.120	0.057	0.125	0.192
	262	0.083	0.035	0.031	0.043	0.138	0.212
	266	0.048	0.035	0.047	0.029	0.050	0.000
	270	0.036	0.023	0.094	0.071	0.063	0.000

	274	0.024	0.105	0.109	0.071	0.063	0.115
	278	0.012	0.140	0.063	0.129	0.025	0.000
	282	0.012	0.035	0.016	0.071	0.050	0.058
	286	0.036	0.035	0.031	0.057	0.063	0.039
	290	0.012	0.000	0.031	0.043	0.000	0.000
	294	0.012	0.012	0.000	0.000	0.000	0.000
	298	0.012	0.012	0.016	0.000	0.000	0.000
	302	0.000	0.000	0.016	0.014	0.000	0.000
	306	0.024	0.000	0.000	0.014	0.000	0.000
	310	0.012	0.000	0.000	0.000	0.000	0.000
	H exp.	0.849	0.874	0.894	0.894	0.883	0.828
	H n.b.	0.859	0.884	0.908	0.907	0.895	0.845
	H obs.	0.667	0.907	0.875	0.800	0.800	0.769
OMM1082							
	168	0.000	0.000	0.031	0.000	0.000	0.037
	176	0.024	0.012	0.000	0.044	0.063	0.000
	180	0.049	0.023	0.078	0.029	0.050	0.074
	184	0.110	0.047	0.156	0.088	0.038	0.019
	188	0.134	0.105	0.109	0.074	0.075	0.056
	192	0.159	0.233	0.250	0.162	0.150	0.111
	196	0.110	0.163	0.109	0.103	0.175	0.333
	200	0.110	0.105	0.047	0.044	0.050	0.167
	204	0.073	0.151	0.094	0.088	0.100	0.000
	208	0.061	0.105	0.047	0.162	0.063	0.074
	212	0.061	0.035	0.047	0.030	0.100	0.074
	216	0.012	0.012	0.016	0.015	0.075	0.037
	220	0.073	0.000	0.016	0.074	0.050	0.000
	224	0.012	0.000	0.000	0.044	0.013	0.000
	232	0.000	0.000	0.000	0.029	0.000	0.019
	240	0.012	0.012	0.000	0.015	0.000	0.000
	H exp.	0.899	0.859	0.866	0.902	0.899	0.826
	H n.b.	0.910	0.870	0.880	0.915	0.910	0.841
	H obs.	0.829	0.861	0.938	0.882	0.900	0.889
OTSG249b							
	127	0.012	0.012	0.000	0.000	0.000	0.000
	131	0.012	0.012	0.016	0.014	0.063	0.077
	135	0.012	0.000	0.000	0.014	0.000	0.000
	139	0.035	0.070	0.016	0.000	0.000	0.019
	143	0.140	0.116	0.063	0.129	0.025	0.039
	147	0.023	0.000	0.016	0.000	0.013	0.019

	151	0.023	0.093	0.047	0.0867	0.038	0.0779
	155	0.093	0.023	0.094	0.157	0.050	0.077
	159	0.093	0.058	0.141	0.071	0.113	0.058
	163	0.105	0.081	0.000	0.086	0.013	0.039
	167	0.174	0.198	0.125	0.114	0.075	0.019
	171	0.149	0.140	0.234	0.143	0.138	0.058
	175	0.012	0.081	0.141	0.114	0.125	0.077
	179	0.081	0.058	0.078	0.014	0.175	0.288
	183	0.023	0.023	0.016	0.014	0.025	0.019
	187	0.000	0.012	0.000	0.000	0.025	0.019
	191	0.000	0.012	0.000	0.000	0.038	0.077
	195	0.000	0.000	0.000	0.014	0.012	0.000
	199	0.000	0.000	0.000	0.000	0.012	0.000
	203	0.000	0.012	0.000	0.014	0.050	0.039
	207	0.000	0.000	0.000	0.000	0.012	0.000
	215	0.000	0.000	0.000	0.014	0.000	0.000
	219	0.023	0.000	0.016	0.000	0.000	0.000
	H exp.	0.8912	0.892	0.868	0.891	0.902	0.877
	H n.b.	0.902	0.903	0.881	0.904	0.914	0.894
	H obs.	0.861	0.954	0.938	0.914	0.825	0.885
OMY1101							
	134	0.000	0.000	0.000	0.000	0.000	0.019
	138	0.024	0.000	0.000	0.000	0.068	0.135
	142	0.000	0.023	0.047	0.000	0.027	0.000
	146	0.012	0.000	0.016	0.000	0.000	0.000
	150	0.107	0.174	0.125	0.086	0.054	0.077
	154	0.071	0.047	0.109	0.100	0.054	0.000
	158	0.119	0.105	0.109	0.186	0.162	0.135
	162	0.250	0.244	0.188	0.186	0.230	0.173
	166	0.036	0.058	0.110	0.100	0.108	0.135
	170	0.095	0.198	0.125	0.100	0.027	0.019
	174	0.048	0.058	0.031	0.086	0.162	0.212
	178	0.071	0.023	0.063	0.057	0.081	0.000
	182	0.036	0.012	0.000	0.014	0.014	0.019
	186	0.024	0.023	0.016	0.029	0.000	0.058

	190	0.024	0.000	0.000	0.000	0.000	0.000
	194	0.024	0.012	0.016	0.014	0.000	0.000
	198	0.036	0.023	0.000	0.029	0.000	0.019
	202	0.024	0.000	0.016	0.014	0.000	0.000
	210	0.000	0.000	0.000	0.000	0.014	0.000
	222	0.000	0.000	0.031	0.000	0.000	0.000
	H exp.	0.884	0.849	0.889	0.881	0.864	0.860
	H n.b.	0.891	0.859	0.903	0.894	0.876	0.877
	H obs.	0.786	0.791	0.875	0.857	0.811	0.885
OTSG85							
	96	0.000	0.000	0.000	0.000	0.013	0.000
	116	0.012	0.012	0.016	0.014	0.013	0.000
	120	0.000	0.000	0.000	0.029	0.000	0.000
	124	0.000	0.000	0.000	0.000	0.038	0.039
	128	0.012	0.023	0.016	0.000	0.000	0.000
	132	0.110	0.023	0.047	0.014	0.025	0.000
	136	0.037	0.012	0.000	0.071	0.025	0.039
	140	0.024	0.023	0.063	0.000	0.038	0.115
	144	0.122	0.035	0.063	0.057	0.125	0.039
	148	0.110	0.093	0.141	0.200	0.075	0.058
	152	0.012	0.081	0.094	0.057	0.025	0.039
	156	0.061	0.070	0.125	0.014	0.088	0.115
	160	0.037	0.093	0.031	0.043	0.038	0.039
	164	0.037	0.058	0.047	0.029	0.075	0.077
	168	0.024	0.047	0.031	0.043	0.063	0.039
	172	0.061	0.047	0.078	0.029	0.050	0.019
	176	0.037	0.116	0.031	0.086	0.000	0.019
	180	0.073	0.035	0.047	0.071	0.013	0.019
	184	0.061	0.081	0.047	0.057	0.113	0.077
	188	0.061	0.081	0.031	0.086	0.050	0.039
	192	0.085	0.035	0.047	0.043	0.000	0.058
	196	0.000	0.012	0.031	0.029	0.075	0.077
	200	0.024	0.023	0.016	0.014	0.025	0.019
	204	0.000	0.000	0.000	0.014	0.013	0.058
	208	0.000	0.000	0.000	0.000	0.025	0.019

	H exp.	0.926	0.932	0.925	0.916	0.930	0.933
	H n.b.	0.937	0.943	0.940	0.929	0.942	0.952
	H obs.	0.927	0.907	0.938	0.857	0.875	0.846
OTSG253c							
	185	0.012	0.036	0.000	0.0294	0.000	0.000
	189	0.012	0.000	0.000	0.0147	0.000	0.000
	193	0.000	0.048	0.000	0.0441	0.013	0.000
	197	0.036	0.000	0.016	0.0000	0.013	0.000
	201	0.012	0.071	0.141	0.0294	0.051	0.039
	205	0.024	0.036	0.078	0.1471	0.026	0.019
	209	0.071	0.048	0.047	0.0147	0.141	0.115
	213	0.012	0.048	0.203	0.0000	0.180	0.135
	217	0.048	0.095	0.110	0.0588	0.090	0.115
	221	0.131	0.083	0.047	0.1029	0.039	0.058
	225	0.036	0.0356	0.031	0.0441	0.090	0.019
	229	0.107	0.095	0.094	0.0294	0.090	0.173
	233	0.024	0.048	0.078	0.0735	0.064	0.000
	237	0.083	0.036	0.000	0.0882	0.026	0.019
	241	0.060	0.071	0.000	0.0147	0.026	0.000
	245	0.060	0.024	0.016	0.0294	0.026	0.058
	249	0.107	0.083	0.000	0.0588	0.026	0.096
	253	0.107	0.036	0.031	0.1029	0.051	0.019
	257	0.024	0.036	0.000	0.0294	0.000	0.000
	261	0.012	0.060	0.047	0.0147	0.000	0.000
	265	0.000	0.000	0.031	0.0588	0.000	0.000
	269	0.000	0.012	0.031	0.0147	0.013	0.058
	273	0.024	0.000	0.000	0.0000	0.013	0.000
	277	0.000	0.000	0.000	0.0000	0.026	0.039
	281	0.000	0.000	0.000	0.0000	0.000	0.039
	H exp.	0.922	0.937	0.895	0.9243	0.908	0.900
	H n.b.	0.933	0.948	0.909	0.9381	0.920	0.918
	H obs.	0.619	0.667	0.813	0.5882	0.769	0.962

Table 5. Observed heterozygosities (H_o) for the 7 microsatellite loci and average of the heterozygosity direct count of each population and each locus. H_o values that deviated significantly from Hardy-Weinberg equilibrium at a significance level of 0.05 are shown with an asterisk.

Loci	W00	W01	S01	W02	S02	Hatch	Locus average
OtsG 83b	0.85	0.91	0.97	0.85	0.71*	0.77	0.84
Omm 1087	0.67*	0.91	0.88	0.80	0.80	0.77	0.80
Omm 1082	0.83*	0.86	0.94	0.88	0.90	0.89*	0.88
OtsG 249b	0.86	0.95	0.94	0.91	0.83*	0.88	0.90
Omy1101	0.79*	0.79	0.88	0.86	0.81*	0.88	0.83
OtsG 85	0.93	0.91	0.94	0.86	0.88	0.85*	0.89
OtsG 253c	0.62*	0.67*	0.81	0.59*	0.77*	0.96	0.74
Population Average	0.79	0.86	0.91	0.82	0.81	0.86	

Table 6. Inbreeding coefficient (F_{IS}) for 7 microsatellite loci in each collection. Values with asterisks represent mean F_{IS} values that are significant with 95% confidence.

	W00	W01	S01	W02	S02	Hatch.
Ots83b	0.085	0.023	-0.028	0.097	0.235	0.171
Omm1087	0.226	-0.026	0.0366	0.120	0.107	0.091
Omm1082	0.090	0.010	-0.0665	0.037	0.011	-0.058
Ots249b	0.047	-0.056	-0.0647	-0.012	0.098	0.008
Omy1101	0.123	0.080	0.0312	0.041	0.075	-0.009
Ots85	0.011	0.037	0.0027	0.078	0.072	0.113
Ots253c	0.339	0.299	0.1079	0.377	0.166	-0.048
mean	0.131*	0.054	0.0032	0.107*	0.120*	0.040

Table 7. Matrix of pair-wise F_{ST} estimates for differentiation at seven loci. P-values are computed using 2000 permutations in GENETIX. *P < 0.05; **P < 0.01; ***P < 0.001.

	Winter 2000	Winter 2001	Summer 2001	Winter 2002	Summer 2002
Winter 2001	0.004				
Summer 2001	0.007**	0.004			
Winter 2002	0.002	0.004	0.005**		
Summer 2002	0.009***	0.011***	0.004	0.010***	
Hatchery 2002	0.021***	0.024***	0.023***	0.027***	0.002

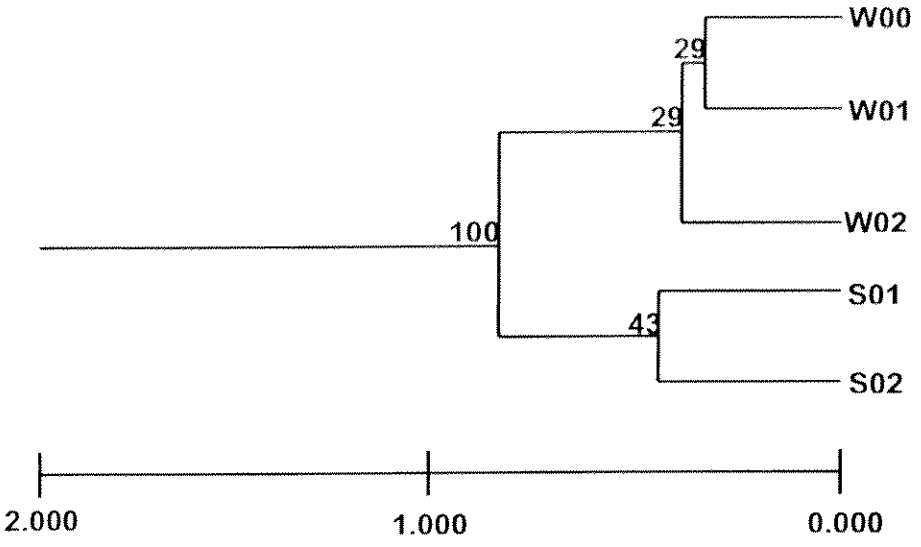
Table 8. Nei's unbiased genetic distance (2000 permutations in GENETIX) above diagonal and identity (Nei 1978; computed with GDA) below diagonal. *P < 0.05; **P < 0.01; ***P < 0.001.

	W00	W01	S01	W02	S02	Hatch
W00		0.014	0.020**	0.016	0.021**	0.035***
W01	0.963		0.016	0.016	0.022***	0.036***
S01	0.918	0.962		0.020 *	0.017	0.037***
W02	0.958	0.953	0.935		0.024***	0.042***
S02	0.890	0.886	0.951	0.869		0.017
Hatch	0.793	0.779	0.784	0.725	0.977	

Table 9. AMOVA comparison of allelic variation from 7 loci between Klamath River summer and winter run steelhead.

Source of variation	d.f.	Variance Component	Percentage of Variation	*P*
between years (2000-2002)	2	-12.73	-0.68%	0.747
among winter and summer run from a single year (2001 and 2002)	1	48.39	2.59%	>0.001
within samples	381	1832.79	97.52%	>0.001

Figure 1. UPGMA tree of Nei's unbiased genetic distance (1978) for winter and summer steelhead samples from 2000-2002. Collections are abbreviated as followed: W=Winter, S= Summer, and years are abbreviated as their last 2 years (2000=00, etc.). Proportion of replicates resulting in similar node strucuture recorded next to node.



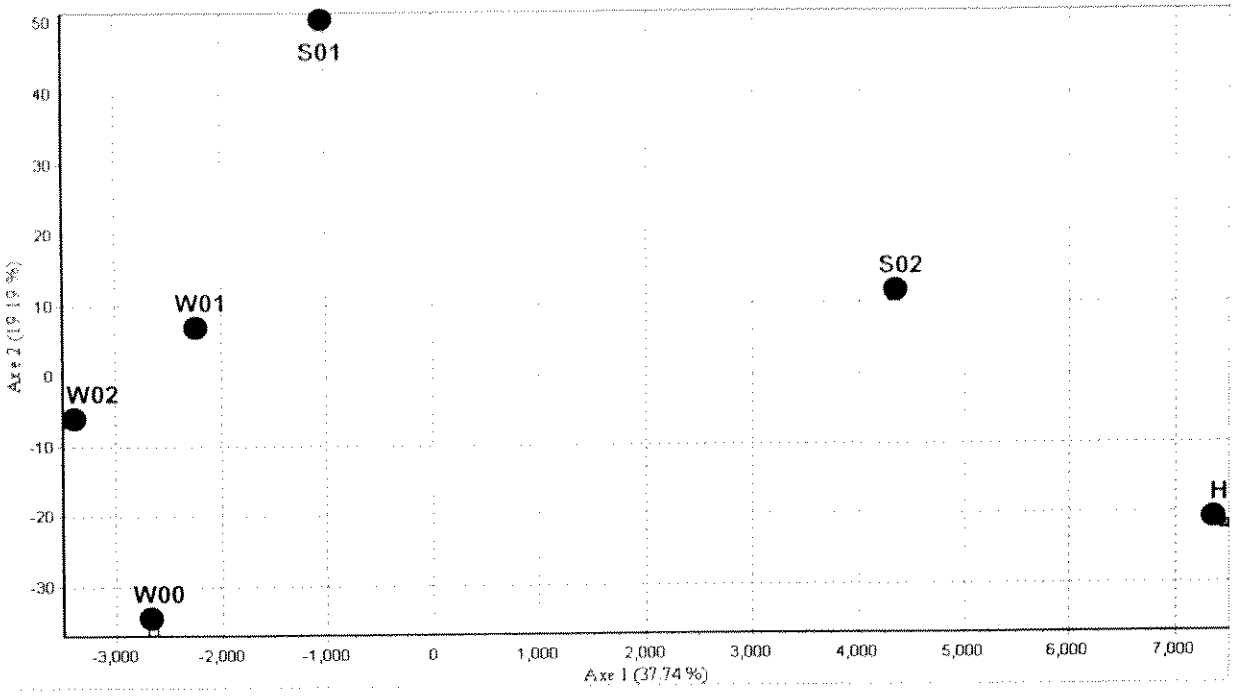


Figure 2. FCA of six samples of steelhead collected from the Lower Klamath River. Collections are abbreviated as followed: W=Winter, S= Summer, and years are abbreviated as their last 2 years (2001=01). Note the proximity of winter samples compared to summer samples.

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Summary of Expenditures

SUBG - GENERAL ASSISTANCE					
		Balance Forward	6,000.00CR	5,964.00	
Period Totals:			0.00	0.00	0.00
Totals for SUBG:	Balance:	36.00CR	6,000.00CR	5,964.00	0.00
SUB3 - SUPPLIES AND EXPENSE					
		Balance Forward	1,417.69CR	2,646.68	
Period Totals:			0.00	0.00	0.00
Totals for SUB3:	Balance:	1,228.99OD	1,417.69CR	2,646.68	0.00
SUB5 – TRAVEL					
		Balance Forward	500.00CR	20.81	
Period Totals:			0.00	0.00	0.00
Totals for SUB5:	Balance:	479.19CR	500.00CR	20.81	0.00
SUB6 - EMPLOYEE BENEFITS					
		Balance Forward	1,500.00CR	786.20	
Period Totals:			0.00	0.00	0.00
Totals for SUB6:	Balance:	713.80CR	1,500.00CR	786.20	0.00
Direct Cost Subtotals:	Balance:	0.00CR	9,417.69CR	9,417.69	0.00
INDR - INDIRECT COSTS					
		Balance Forward	4,592.31CR	4,592.31	
Period Totals:			0.00	0.00	0.00
Totals for INDR:	Balance:	0.00	4,592.31CR	4,592.31	0.00
Period Totals for 3-ANS716Y			0.00	0.00	0.00
Totals for 3-ANS716Y	Balance:	0.00CR	14,010.00CR	14,010.00	0.00